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SHORT COMMUNICATION

TAURINE ACCUMULATION BY THE HEART OF EMBRYONIC SKATES, *RAJA EGLANTERIA*

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The hearts of elasmobranchs, like those of other vertebrates, contain high concentrations of the β-amino acid taurine, which is thought to play an osmoregulatory role in cardiac tissue. Although mammals and certain invertebrates can synthesize taurine from sulfur-containing precursors, e.g. cysteine, fish are unable to do so since they lack at least one enzyme involved in taurine biosynthesis: cysteine sulfinate decarboxylase (Jacobsen and Smith, 1968). Therefore, taurine must be acquired by fish *via* their diet (King *et al.* 1986). This poses a problem for the embryos of those fish that lay eggs and, for the early period of development at least, have no direct contact with amino acids in the external environment. Although solutes in the egg-case fluids can exchange with the sea water (Evans, 1981), the taurine concentration gradient between the egg case and the environment actually favors outward movement of taurine rather than uptake (L. Golstein, unpublished results). Therefore, we chose to study the mechanism by which the embryonic skate heart accumulates and maintains elevated concentrations of taurine in its heart. Concentrations of about 50 mmol l^{-1} taurine are typically found in cardiac muscle of adult specimens of the little skate *Raja erinacea* (Boyd *et al.* 1977).

Adult clearnose skates (*Raja eglanteria* Bosc) were captured passively offshore at Sarasota, Florida, with nets. Freshly laid eggs from these fish were obtained from a breeding colony maintained at the Mote Marine Laboratory, Sarasota, FL. This allows precise dating of the post-deposition age of each developing embryo as well as knowledge of its history. Adult skates were maintained in a controlled-environment, recirculating seawater tank at the laboratory. Water temperature ranged from 20 to 22 °C and salinity from 30 to 35 ‰. Skates were checked daily and the dates noted when eggs were laid. The eggs were incubated under the same conditions described for adult skate maintenance. Embryos were used 23–28 days after deposition (Fig. 1), before the respiratory canals in the egg-case horns became unplugged (Luer and Gilbert, 1985). Any eggs in which the respiratory canals had become unplugged, thus allowing sea water to enter and leave the egg case, were rejected.

Key words: osmoregulation, amino acids, elasmobranchs.

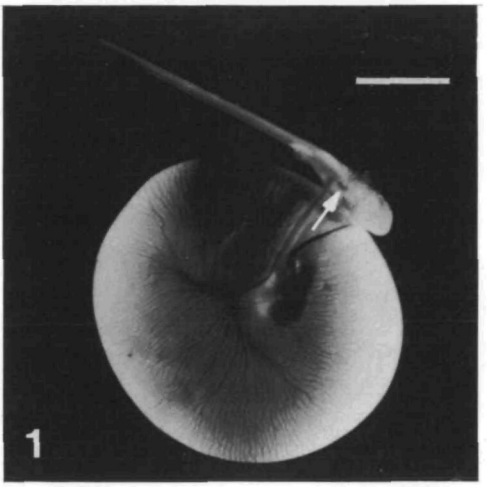

Fig. 1. Clearnose skate (*Raja eglanteria*) embryo 24 days after egg deposition. Developing cardiac tissue can be seen as the dark area *indicated by the arrow*, just anterior to the site of yolk-stalk attachment on the ventral surface of the embryo. (Total length of embryo is 4.0 cm.)

Embryos were removed from their egg cases by carefully cutting a window through one of the flat sides of the leathery case. They were then separated from their yolk sacs by severing the yolk stalks. Hearts were excised, free of as much non-cardiac tissues as possible, using iridectomy scissors and fine-tipped forceps. The cardiac tissue was weighed and placed directly into incubation or homogenizing medium. Yolk was drained from the egg cases and weighed.

In the transport and metabolism studies, hearts were placed in 1.0 ml of elasmobranch incubation medium (Forster and Hannafin, 1980) in porcelain depression slides to maximize surface-to-volume ratio and oxygenation of the medium. The incubation medium contained either 0.1 mmol l^{-1} [^{14}C]taurine (0.5 μCi ml^{-1}) or 0.1 mmol l^{-1} [^{14}C]cystine (2.0 μCi ml^{-1}); (N.E. Nuclear). Incubation was carried out at approximately 25 °C. After incubation the hearts were removed, homogenized in 10 % sulfosalicylic acid (SSA) and centrifuged. Supernatants from hearts incubated in [^{14}C]taurine were assayed for ^{14}C by liquid scintillation counting. Supernatants from hearts incubated with [^{14}C]cystine were added to the separation column of an automatic amino acid analyzer (Boyd *et al.* 1977) and the fractions in which taurine eluted (identified with taurine standard) were assayed for ^{14}C by liquid scintillation counting. In addition, supernatants from some hearts were assayed for non-radioactive taurine by automatic amino acid analysis.

Yolk was homogenized with 4 vols of $CHCl_3/CH_3OH$ (2:1). Next, 2 vols of 20 % SSA was added and the mixture homogenized. Then 2 vols of $CHCl_3$ was added and homogenized. The homogenate was centrifuged and the supernatant removed

and dried under vacuum at 40°C. The residue was dissolved in 0.25 vols of H_2O and analyzed for taurine by automatic amino acid analysis.

Skate hearts taken from embryos 24–26 days after eggs had been laid had taurine concentrations of $24\pm5\,\text{mmol}\,g^{-1}$ cardiac tissue [mean±S.E. (N=4)]. Assuming that the taurine concentration in the blood of embryonic skates is of the same order as that in adult skates (about $0.1\,\text{mmol}\,l^{-1}$, Boyd *et al.* 1977), the embryonic hearts had heart/blood taurine concentration ratios similar to those observed in adult skates. Therefore, we examined the source of cardiac taurine in embryonic skates.

Taurine can be synthesized from metabolic precursors, such as cysteine, in some animals (Jacobsen and Smith, 1968). However, fish tissues do not seem to have this capacity, at least when adult, probably because of the lack of a key enzyme in taurine biosynthesis, cysteine sulfinate decarboxylase. Nevertheless, we tested the ability of embryonic skate hearts to convert [^{35}S]cysteine to taurine. Embryonic skate hearts (23–28 days) were incubated with [^{14}C]cystine, which is reduced to [^{14}C]cysteine by the tissues. Taurine was isolated from extracts of these hearts and assayed for ^{14}C. No incorporation of label from cystine into taurine was found. Thus, the embryonic skate heart, like that of the adult, does not appear to have the ability to synthesize taurine from metabolic precursors.

We next examined the ability of the embryonic heart to accumulate taurine from extracellular sources. Embryonic hearts (age range 23–28 days after oviposition) incubated for 3 h in medium containing $0.1\,\text{mmol}\,l^{-1}$ [^{14}C]taurine accumulated taurine to levels which were 5.4±0.6 (N=7) (mean±S.E.) times greater than the concentration in the medium. Forster and Hannafin (1980) reported that atrial strips of adult skate (*R. erinacea*) heart incubated in $0.1\,\text{mmol}\,l^{-1}$ [^{14}C]taurine concentrated the amino acid to 2–3 times that in the medium during 3 h of incubation. Thus, the embryonic heart at 23–28 days has a taurine transport capacity at least as active as that found in adult skates.

The question next arose as to the source of taurine in the skate egg that could supply the amino acid to the heart and other tissues of the embryo. The largest source of nutrients in the egg is the yolk mass. Skate egg yolk was found to have a taurine concentration of $26\pm2\,\text{mmol}\,l^{-1}$ (N=4) at 25–26 days incubation. The total taurine content of the yolk was 174±17 μmol. Skate embryos at this age have an average heart mass of 7±1 mg (N=3), which is about 4 % of the body mass. Since the cardiac taurine concentration averages $24\pm5\,\text{mmol}\,l^{-1}$ (N=4) at this age, the total cardiac taurine content is 0.17±0.04 μmol. Thus, up to 25 days after the eggs are laid the yolk contains more than enough taurine to supply the heart (and other tissues, e.g. brain). Since the yolk taurine content is so large compared with the needs of the heart, there is probably more than sufficient taurine in the yolk to supply the heart until hatching at 82±4 days (Luer and Gilbert, 1985).

In conclusion, embryonic skate hearts contain significantly elevated concentrations of taurine by 23–28 days of incubation. It appears that the accumulated taurine is not synthesized in the cardiac cells but rather is accumulated *via* active

transport from the extracellular fluid. The egg yolk contains sufficient taurine to serve as a reservoir for this amino acid during the entire incubation of the egg.

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